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Pancreatic Head Mass, What Can Be Done?
Diagnosis: Laboratory

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In the field of laboratory medicine, efforts are being made to provide clinicians with evidence conducive to correct clinical decision-making in patients with pancreatic diseases. In patients with pancreatic head mass, the diagnosis can only be made by deciding whether or not pancreatic cancer is present. The diagnostic work-up may start with the study of different biological samples, including sera and/or urine which are easy to collect, unlike pancreatic juice, bile or pancreatic tissue samples, which require invasive techniques, and are therefore less widely used. The serological diagnosis of pancreatic head mass depends mainly on the use of tumor markers, which must have a sensitivity and a specificity of almost 100% if they are to be clinically effective, allowing a differential diagnosis between cancer and benign diseases of the pancreas (which are mainly chronic pancreatitis). Several families of molecules have been studied as possible tumor markers, including oncofetal antigens (CEA and POA), pancreatic enzymes, blood group-related antigens and, more recently, oncogenes and tumor suppressor genes [1, 2]. Neither pancreatic enzymes nor CEA are useful in clinical practice, since they have a sensitivity and specificity of below 50%. Blood group related antigens, CA 19-9 in particular, have the highest diagnostic efficacy in distinguishing between pancreatic cancer and chronic pancreatitis. Figure 1 shows the biochemistry of the Lewis blood group-related antigens [3].

ANTIGEN	STRUCTURE
Le^a	Galb1-3GlcNAcb1-3Gal- | a1-4 Fuc
Sialyl-Le^a (CA19-9)	SAa2-3Galb1-3GlcNAcb1-3Gal- | a1-4 Fuc
Le^b	Galb1-3GlcNAcb1-3Gal- | a1-2 | a1-4 Fuc Fuc
Le^x	Galb1-4GlcNAcb1-3Gal- | a1-3 Fuc
Sialyl-Le^x	SAa2-3Galb1-4GlcNAcb1-3Gal- | a1-3 Fuc
Le^y	Galb1-4GlcNAcb1-3Gal- | a1-2 | a1-3 Fuc Fuc

Figure 1. Blood group related antigens

The sensitivity and the specificity of CA 19-9 in diagnosing pancreatic cancer range from 70 to 95% and 72 to 90% respectively. Although most pancreatic cancers cause an increase in serum CA 19-9 levels, this tumor marker does not approach 100%. This results from two main pathophysiological aspects: 1) since individuals with Lewis a-/b- status (7-10% of the general population) cannot synthesize sialyl Lewis a antigenic determinant (Figure 1), even if they have a large tumor they will have low circulating CA 19-9 levels; 2) the

release of CA 19-9 antigen in cell culture media is correlated with the number of neoplastic cells in culture, and this phenomenon is reflected "in vivo" by the association between CA 19-9 levels and the tumor stage. The clinical effect of this is that CA 19-9 has a low sensitivity in the diagnosis of circumscribed tumors and when used for screening programs. Regarding specificity, the lack of absolute results is due to several factors: 1) tumors of non-pancreatic origin may cause an increase in CA 19-9 serum levels (biliary: sensitivity 55-79%; hepatocellular and cholangiocellular: sensitivity 22-51%; gastric; colorectal; ovarian; lung; breast and uterine); 2) benign diseases of the pancreo-biliary tree (chronic pancreatitis and obstructive jaundice) may also cause significant increases in serum CA 19-9. An altered hepatic function, whether caused by cancer or a benign disease, may give rise to increased serum CA 19-9 levels, due to its reduced molecular clearance, which occurs mainly through the hepatic metabolism [4].

CA 19-9 may also be useful in monitoring pancreatic cancer since it correlates closely with the clinical course of the disease after surgery and chemotherapy and/or radiotherapy. It reaches normal levels within 2 to 4 weeks after radical surgery: there is a transient decrease after successful palliative therapy and an increase before clinical relapse [2, 5].

In this era of molecular biology, numerous oncogenes and tumor suppressor genes have been described, several of which are often altered in patients with pancreatic cancer. The genes most frequently found to be altered in patients with pancreatic adenocarcinoma are K-*ras*, p53, p16 and they are deleted in pancreatic carcinoma-locus 4 (DPC4) [6]. More than 90% of pancreatic tumors bear codon 12 K-*ras* point mutations. To our knowledge, this is the highest frequency to be reported for any tumor type. Such mutations have been observed in the early phases of pancreatic carcinogenesis, determining the synthesis of an altered p21 protein [3, 4]. Normal p21 shifts from an active (bound to GTP) to an inactive (bound to GDP) state via its intrinsic GTPase activity and via its sensitivity to the activity of GTPase activating protein (GAP). The transformed p21 becomes insensitive to GAP, thus leading this protein to a constitutive and permanent activation stimulating cell growth [7-9]. Another gene frequently altered in pancreatic cancer is p16 (homozygously deleted in about 40% of pancreatic carcinomas), an inhibitor of cyclin-dependent kinase 4 (CDK4) which promotes the progression of the cell division cycle through the late G1 phase to G1/S [10]. The genetic alterations involving p16 may be the methylation of the promoter region (15% of cases), homozygous deletion (40%) or deletion accompanied by intragenic mutations in the other allele (40%). The tumor suppressor gene p53, which controls the cell cycle and induces apoptosis, is altered in 50 to 70% of different types of pancreatic cancer, and is usually inactivated by the loss of one allele accompanied by a intragenic mutation of the other allele [10, 11]. The gene DPC4 is altered in about 50% of pancreatic tumors, and encodes a protein belonging to the SMAD family, which is involved in the nuclear translocation of the inhibitory signal for cell growth starting from the interaction between transforming growth factor (TGF) beta1 and its membrane receptor, TGF-beta1R [10, 12, 13].

The above-mentioned genes have been studied in tissue samples and some, K-*ras* in particular, have also been evaluated in biological samples (not only pancreatic tissue) in order to enable the identification, in easily obtained samples, of gene alterations indicating the presence of a pancreatic tumor. K-*ras* point mutations have been studied in duodenal juice, in stools and in serum samples [14-21]. Overall, its sensitivity in the detection of pancreatic tumors decreases when a search is made for K-*ras* in samples other than from the tumor tissue (Table 1).

Table 1. The detection of pancreatic cancer: sensitivity and overall specificity of K-*ras* point mutation identification in different samples.

Sensitivity Specificity	
Tumor tissue	71-100%
Duodenal juice	25-100%
Stool	55%
Serum	0-60%
Overall	66-100%

In laboratory medicine, serum and/or stools should be satisfactory for use as samples for diagnostic purposes, since they are easily obtained, thus favouring patient compliance. However, the sensitivity of K-*ras* point mutation identification is unsatisfactory in diagnosing pancreatic cancer using these samples, perhaps partly due to methodological problems (presence of Taq polymerase inhibitors in faecal samples and low amounts of mutated K-*ras* in a wide background of normal serum K-*ras*). It cannot therefore as yet be used in clinical practice, although it may be routinely used in the future, in line with improvements in molecular biology techniques.

The identification of lymph node or distant metastasis, routinely made with histology on surgical specimens, is a crucial step in staging gastrointestinal tumors, pancreatic cancer in particular. In recent years, in the attempt to improve upon the sensitivity of histology in identifying lymph-node metastasis, various techniques have been studied, including immunocytochemical analysis, with antibodies elicited towards cancer-specific antigenic determinants, and the molecular detection of cancer cells [22]. The latter approach has received particular attention, not only in the identification of lymph-node cancer cells, but also in their identification in blood and bone marrow [23-27]: as the molecular approach is highly sensitive, it is of great potential in the identification of scarce cancer cells (1 cell in a population of 10^7-10^8). The molecular detection of cancer cells in a population of benign cells must target molecular determinants commonly expressed by tumoral, but not by non-tumoral, cells. Among these determinants, considering tumors of epithelial origin, such as those of the gastrointestinal tract, are the mRNA encoding for CEA which are not usually expressed by normal lymph nodes, circulating and bone marrow cells [22-27]. It has been suggested that CEA mRNA detection in lymph nodes, blood and bone marrow is of prognostic value in patients with colorectal and pancreatic cancer, since it probably indicates the presence of micrometastatic foci that cannot be identified by microscopy [28].

In the diagnosis of pancreatic head tumors, diabetes mellitus has received particular attention. This metabolic alteration is, in fact, associated with pancreatic cancer in more than 80% of cases, and in some it is characterized by reduced glucose tolerance and reduced insulin secretion [29-32]. There is a body of clinical and experimental data demonstrating that pancreatic-cancer-associated diabetes mellitus is due to the cancer itself rather than pancreatic islet destruction. It has been suggested that soluble mediators released by pancreatic cancer cells play a role in interfering with the metabolism of glucose. Pancreatic-cancer-associated diabetes mellitus or glucose intolerance are improved, or cured, following surgical removal of the pancreatic mass [33]. This indicates that diabetes mellitus is not correlated with islet cell destruction, but to the presence of the tumor itself. Furthermore, in pancreatic cancer patients, the release of insulin after glucagon stimulation indicates a reduced beta cell function [34]. In agreement with this clinical data, the treatment of isolated islets of Langerhans with pancreatic cancer conditioned media dissociates insulin from amylin secretion [35]. Moreover, it has been demonstrated in clinical series that besides the presence of an altered function of Langerhans islets, there is also a peripheral insulin resistance [32, 33]. In this respect it has been demonstrated that pancreatic tumor extracts determine an altered glycogen synthesis in isolated rat muscles [36]. Furthermore, pancreatic cancer conditioned media can also induce fasting hyperglycaemia in SCID mice [37] as well as inhibiting

hepatic glycolysis, thus possibly favouring the synthesis of tryglycerides [38, 39]. It has been suggested that, one of the possible pancreatic cancer-associated diabetogenic products, soluble low molecular weight peptides play a role [39, 40]. A further valuable laboratory tool in the diagnosis of pancreatic head mass might be the identification of this/these substances and their determination in serum or urine.

Key words Diabetes Mellitus; Genes, Suppressor, Tumor; Oncogenes; Pancreatic Neoplasms; Diagnosis; Tumor Markers, Biological

Abbreviations CDK4: cyclin-dependent kinase 4; CEA: carcinoembryonic antigen; DPC4: deleted in pancreatic carcinoma-locus 4; GAP: GTPase-activating protein; POA: pancreatic oncofetal antigen; TGF: transforming growth factor

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